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Date: July 14, 2008 Rebecca A. Bellas

Rebecca A. Bellas

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application

Applicant:	Kunishima	:	Art Unit:	1624
Serial No.:	10/588,940	:	Examiner:	Venkat Balasubramanian
Filed:	August 8, 2006	:		

Title: DEHYDRATING CONDENSATION AGENT HAVING PROPERTY OF ACCUMULATING AT INTERFACE WITH WATER

RULE 132 DECLARATION

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Munetaka Kunishima, declare and say as follows:

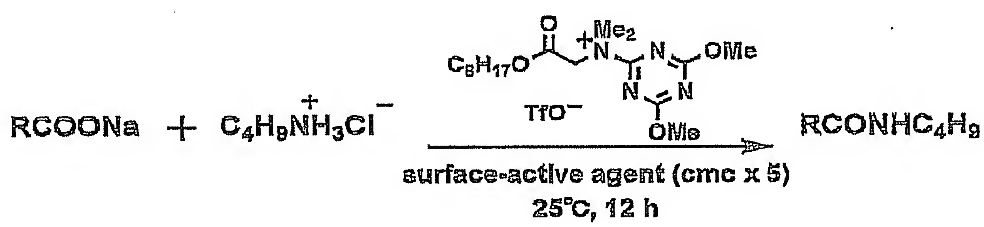
I am the inventor of the invention described in the above-identified U.S. patent application and, therefore, I am thoroughly familiar with the subject matter of the invention. I am a professor of Faculty of Pharmaceutical Sciences, Kanazawa University, since March 2008. I studied Organic Chemistry at Kyoto University and

received Ph.D. degree of pharmaceutical Sciences from Kyoto University in 1990. I was appointed to an assistant professor of Faculty of Pharmaceutical Sciences, Kobe Gakuin University in 1990. I was promoted to Associate professor in 2003, and to Professor in 2007, and moved to Kanazawa University in 2008. I joined a research project of PRESTO (Precursory Research for Embryonic Science and Technology), JST (Japan Science and Technology Agency), in research area of "Conversion and Control by Advanced Chemistry," from 2002 to 2005. In 2007, I received The PSJ Award for Divisional Scientific Promotions from the Pharmaceutical Society of Japan (PSJ). My research interests involve dehydrating condensation in various reaction fields and related technologies.

<u>Experiments</u>

Experiment I. Amidation

I-1. Micelle effect on condensation of hexanoate, octanoate, or benzoate



Reaction condition:

Sodium carboxylate	5 mM
n-Butylamine hydrochloride	20 mM
Interface-accumulating condensing agent	1.5 mM
Phosphate Buffer (pH 8)	20 mM

Surfactant (10 times critical micelle concentration (cmc))

SDS 40 mM

Triton X-100 1.5 mM

Table 1: Yield of dehydrating condensation in the presence or absence of surfactant

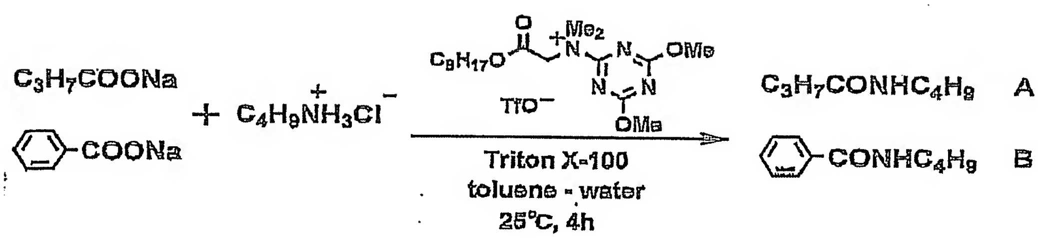
Surfactant	Hexanoate	Octanoate	Benzoate
Without surfactant	7%	8%	8%
SDS	23%	44%	18%
Triton X-100	25%	44%	31%

As seen from Table 1, in no surfactant system (i.e., in a homogeneous system), the yields of resultants are as low as less than 10%. It is found that in such a concentration of the substrate, any carboxylates do not form molecular aggregate phase such as micelles. Therefore, in the homogeneous system, this result is understood that condensation between two molecules is slow because the substrate concentration in the solution system is low. On the contrary, in micelle system to which a surfactant is added, condensation was accelerated and the yield of the product was increased. The yield is correlated with lipophilicity of carboxylate. The difference between the yields of the present system and the homogeneous system cannot be expected. The increased rate of condensation of the present invention is caused by the accelerating effect at a water interface.

I-2. Emulsion effect

In this section, it will be demonstrated that the condensation is further accelerated by adding an organic solvent such as toluene to a reaction system to form emulsion, compared to the addition of surfactant alone.

I-2-1. Emulsion effect on competitive condensation of benzoate and butyrate



Under the same conditions as the experiment described above, the competitive reaction was performed by using equal molar concentration (5mM) or benzoate and butyrate.

Table 2: Reaction in emulsion system

	Triton X-100	Toluene	Yield	Selectivity (A:B)
Solution system	0 mM	0%	10%	45:55
Micelle system	1.5 mM	0%	37%	15:85
Emulsion system	1.5 mM	1.5%	66%	5:95

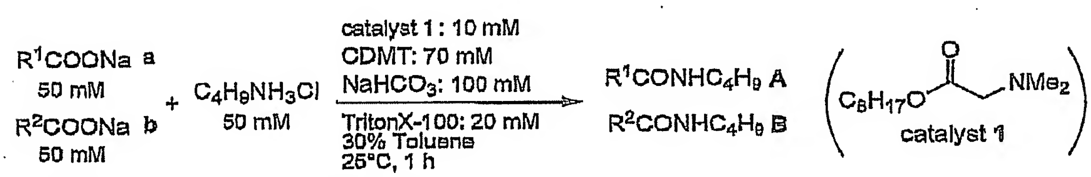
As seen from Table 2, in the solution system where molecular aggregate is not formed, the yield was low and the selectivity was not found because the reaction was not accelerated for both substrates. On the contrary, in the micelle system, the yield and selectivity in benzoate which is highly lipophilic were improved. In the emulsion system,

both yield and selectivity were relatively higher than in micelle system. As shown by this result, it is also found that increased rate of condensation is caused by the interface effect using a dehydrating condensing agent having property of accumulating at interface.

I-2-2. Interface effect of emulsion on the carbon-chain length of fatty acids and the acceleration of reaction

For carboxylic acids from butyrate (C_4) to decanoate (C_{10}), the effect of the carbon-chain long of fatty acids on the reaction selectivity was investigated. The reaction was performed by the method for producing the dehydrating condensing agent having property of accumulating at interface, in which the agent was produced from catalyst 1 that has property of accumulating at interface and CDMT within the reaction system (i.e., at the water interface). As shown in Table 3, two carboxylates a and b, which have different carbon numbers respectively, were competitively reacted in the emulsion system, and then the yields and selectivity were examined.

Table 3: Evaluation of the difference of carbon number and the reaction selectivity by interface effect in emulsion



R^1, R^2 = C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}

Run	a Carboxylic acid (R^1)	$logP_a$	b Carboxylic acid (R^2)	$logP_b$	Yield (%)	A:B Predicted	A:B Measured
1	C_3H_7	0.79	C_4H_9	1.3	75%	24:76	24:76
2	C_4H_9	1.3	C_5H_{11}	1.92	70%	19:81	25:75
3	C_5H_{11}	1.92	C_6H_{13}	2.54	73%	19:81	26:74
4	C_6H_{13}	2.54	C_7H_{15}	3.05	83%	24:76	27:73
5	C_7H_{15}	3.05	C_8H_{17}	3.52	74%	25:75	34:66
6	C_8H_{17}	3.52	C_9H_{19}	4.09	78%	21:79	39:61

In Table 3, logP is a scale that represents lipophilicity of compound by octanol-water partition coefficient. For example, in butyrate ($R=C_3H_7$), octanol-water partition ratio is 6.2/1.0, so that logarithm is 0.79 (log6.2=0.79).

It is thought to be impossible to distinguish the difference in one carbon length between the substrates in a general homogeneous solution system. Therefore, in this experiment, it was found that the carboxylate having more carbon atoms preferentially reacted with amine at the ratio of 4:1 to 3:1. The production ratio was in agreement with the value predicted from logP.

Based on the measured value, the selectivity of hexanoate, heptanoate, the octanoate on butyrate is predicted as 10:90, 4:96, and 1:99, respectively. So, the competitive reaction was performed under the same condition as described above. The results are shown in Table 4 below. The measured value was in agreement with the predicted value, and also the value predicted from logP. Therefore, the reaction selectivity is brought about interface effect based on the difference in lipophilicity, that is, the ratio of carboxylate incorporated into emulsion.

Table 4: Competitive reaction between carboxylates

C_3H_7COONa **a** 50 mM + $C_4H_9NH_3Cl$ 50 mM $\xrightarrow[\text{TritonX-100: 20 mM, 30\% Toluene, 25°C, 1 h}]{\text{catalyst 1: 10 mM, CDMT: 70 mM, } NaHCO_3\text{: 100 mM}}$ $C_3H_7CONHC_4H_9$ **A**

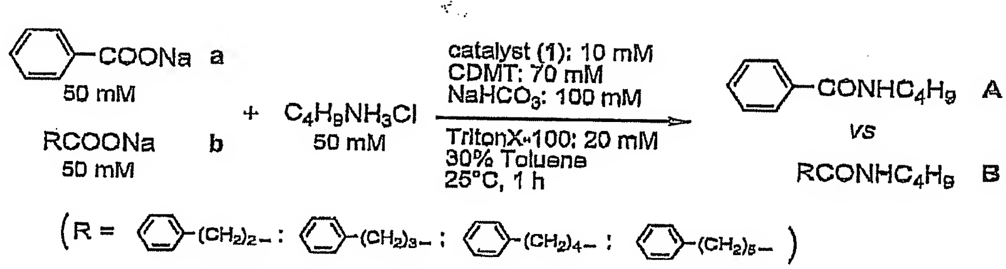
$RCOONa$ **b** 50 mM → $RCONHC_4H_9$ **B**

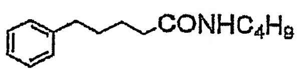
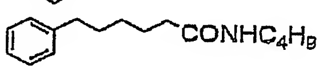
($R = C_5H_{11}, C_6H_{13}, C_7H_{15}$)

run	A	B	yield	selectivity A : B	predicted from measured	predicted from logP
1		 $CONHC_4H_9$	70%	9 : 91	10 : 90	7 : 93
2	 $CONHC_4H_9$	 $CONHC_4H_9$	76%	3 : 97	4 : 96	2 : 98
3		 $CONHC_4H_9$	78%	1 : 99	1 : 99	0.5 : 99.5

Furthermore, as shown in Table 5 below, it was confirmed that in the compound having benzene ring, the longer carbon chain is, the faster the condensation proceeds.

Table 5: Evaluation of the difference of carbon number and the reaction selectivity



A	B	yield	A : B predicted	A : B measured
		79.5%	9 : 91	20 : 80
		67%	3 : 97	12 : 88
		81%	1 : 99	7 : 93
		84%	0.3 : 99.7	2 : 98

Experiment II. Esterification

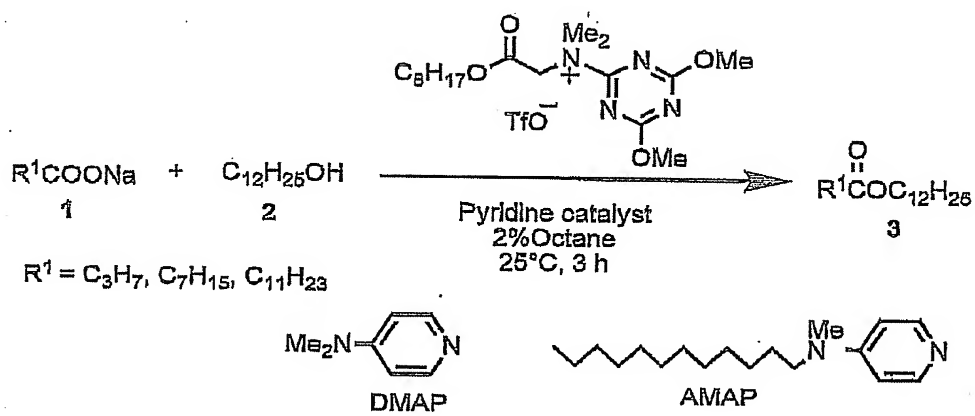
In dehydrating condensation by using DMT-MM, esterification has proceeded at a high yield only in Fischer-type reaction under basic conditions in which alcohol is used as a solvent. However, in aqueous solution, for example, in a solution containing amide, esterification could not proceed. This is because the nucleophilicity of amine is dramatically higher than that of water (twenty thousand times or more reaction ratio), but that of alcoholic hydroxyl group is similar to that of water. Thus, esterification in an aqueous solution has not been performed with any success by any conventional methods.

The present inventor has firstly discovered that an ester can be produced even if water is used as a main solvent, based on the effect of accelerating reaction at a water interface. The experimental results are shown in Table 6.

Reaction condition:

Sodium laurate	15 mM
Dodecanol	15 mM
Condensing agent	1.5 mM
Pyridine catalyst	0.5 mM

Table 6:



Run	R^1	Pyridine	Yield
1	C_3H_7	——	6%
2	C_3H_7	DMAP	5%
3	C_3H_7	AMAP	41%
4	C_7H_{15}	——	29%
5	C_7H_{15}	DMAP	59%
6	C_7H_{15}	AMAP	82%
7	$C_{11}H_{23}$	——	24%
8	$C_{11}H_{23}$	DMAP	69%
9	$C_{11}H_{23}$	AMAP	100% a)

a)10 min

The dehydrating condensation was accelerated in emulsion system where 2%(v/v) of octane was added to the reaction mixture. As seen from Table 6, the yield was dependent on the length of carbon-chain of carboxylates. By addition of pyridine, the reaction of active intermediate with alcohol was accelerated. When amphiphilic pyridine catalyst AMAP that has a long chain alkyl group on the amino group at 4-position was used, the reaction was most accelerated. In the combination with laurate, the reaction was performed at 100% of yield within only 10 minutes.

Furthermore, it was found that the carbon-chain length of alcohol is also important. In the reaction of Table 6, run 9, when butanol (C_4) was used instead of dodecanol, the yield was only 16%.

All results described above demonstrates that the reaction is accelerated only when all components involved in the reaction are amphiphilic, that is, carboxylate and alcohol as substrates, as well as dehydrating condensing agent and pyridine catalyst, are amphiphilic. This is realized by the effect of interface by using amphiphilic dehydrating condensing agent. Therefore, such an accelerating effect on dehydrating condensation would not have been expected from conventional solution system.

I, Dr. Munetaka Kunishima, hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued therein.

M. K[illegible]

Munetaka Kunishima

7 / 01 / 2008

Date